Protocol for Surveillance of initial drug-resistant HIV-1 among ART naive children < 18 months of age newly diagnosed with HIV

World Health Organization

Objectives

1. To describe the prevalence of initial NNRTI and NRTI resistance in newly-diagnosed children < 18 months receiving EID for whom previous ARV exposure is recorded as “known”, “none” or “unknown.

2. To evaluate the impact of PMTCT scale up on the pattern of resistance acquired by infants acquiring the infection despite PMTCT

3. Inform the identification of the best strategy to treat HIV infected infants (LPV/r vs NVP-based or “switch strategy”)

Hypothesis

As ARV use for PMTCT increases, there will be a relatively small proportion of children who become infected with HIV despite PMTCT prophylaxis. However, among those infected, an increasing proportion will harbor drug-resistant HIV strains.

Survey Design

Retrospective cross-sectional survey of DR-HIV prevalence among children diagnosed with HIV by Early Infant Diagnosis (EID) [PCR] methodology using remnant DBS specimens.

Data will be abstracted from laboratory requisition forms that accompany DBS samples

- No identifying information will be collected; the analysis will be unlinked and anonymous.
- Results will not be returned (genotyping will be performed six months to a year after children should already have started ART)

Survey Investigators

- Subgroup of the national HIV drug resistance working group (HIVDR-WG), convened by the Ministry of Health to develop an HIV drug resistance prevention and assessment strategy.

- The HIVDR-WG generally includes all national and international partners involved in national ARV care, HIV surveillance, ARV monitoring and evaluation, PMTCT, relevant laboratory experts and HIVDR researchers.

- WHO staff and CDC staff in-country, and at the regional and central levels, will provide technical support and provide training for implementation and analysis.

Inclusion Criteria

- DBS collected from a **child < 18 months of age who received testing for EID**.
- The DBS specimen tested HIV-positive by DNA PCR.
- At **least one viable remnant DBS** was available if not required for clinical testing or quality assurance (Two-four DBS would be optimal)
- DBS specimen has been **no more than 30 days at room temperature**, then stored at -20°C or -80°C with no thawing before genotyping will be performed.

Exclusion Criterion

- Child is receiving three or more ARV drugs for the purpose of treatment of HIV (rather than prophylaxis to prevent HIV infection) at time of blood draw.

Required Variables

- Date of birth; if not available, age of child in months at time of blood draw
- Gender
- Site name where DBS was collected
- Site type where DBS was collected (e.g., ANC/MCH site, VCT site, pediatric clinic, pediatric hospital)
- Date of DBS collection
- Child receiving ARVs for his/her own treatment (not PMTCT) at time of specimen collection (yes/no)
- Date of first freezing DBS at -20 C or -70 C
- Date of PCR assay

Highly desirable optional variables

- Is child breastfeeding at time of specimen collection?
- ARVs received by mother for PMTCT or maternal health: antepartum; intrapartum; postpartum-during breastfeeding; none: circle those that apply
- ARVs received by child for PMTCT (Yes/No)
- ARVs received by mother sd-NVP; sd-NVP + ZDV; ZDV + 3TC; or other [specify]; a three-ARV regimen (for maternal health or prophylaxis) [specify regimen]
- ARVs received by child for PMTCT: sd-NVP; extended NVP (that is, NVP daily for more than one day); extended ZDV (that is, ZDV daily for more than one day); extended NVP + ZDV (that is, NVP + ZDV daily for more than one day); other [specify]

Contributing sites

- PMTCT, Maternal and Child Health (MCH) or Antenatal (ANC) clinics providing HIV-testing as part of routine follow-up of children < 18 months of age.

- Hospitals or other medical facilities providing HIV testing to symptomatic children < 18 months of age.

- Provider initiated testing and counseling (PITC) sites or Voluntary Counseling and Testing (VCT) sites providing HIV testing for children < 18 months of age.

Participating laboratories

- Ideally, each lab in the country performing child EID will participate and will contribute to the overall sampling (feasible in countries with a limited number of diagnostic laboratories).

Case Definition

Drug-resistant HIV

(Standard sequencing to detect quasi-species present at 20% or higher. *Only the RT region of the HIV genome will be sequenced***)**

-Any mutation or combination of mutations that produce low, intermediate, or high level resistance to a relevant ARV drug or drug class according to the latest Stanford HIVDR database scores

-A Stanford classification of "potential" drug resistance will not classified as drug resistance for the purpose of this survey.

Fasta files representing sequences will be imported into the database, so mutations at all positions will be available for analysis

Sample Size calculations based on:

- "true DR-HIV prevalence" of 50%
- 95% confidence intervals (CI) +/- 7%
- Power = 0.80
- non-amplification rate of 20%
- *These "conservative" assumptions yield the largest sample size and the most precise estimates of prevalence with the most narrow confidence intervals*

Sample Size Examples

In Zimbabwe there is one EID laboratory:

In countries where only one laboratory participates, **the sample size will be 245**.

In Uganda there are eight EID laboratories:

We use a design effect of 2 if more than one laboratory participates. The final effective sample size if more than one laboratory participates is **245 x 2 = 490**

Sampling Strategy

- 1. PPS
- 2. SRS

Specimen handling, transport, and storage

- The survey will use remnant DBS available after all diagnostic, clinical, and quality assurance tests have been performed

- National or site-based guidance should be followed for collecting DBS for HIV diagnosis by PCR.

- For amplification and genotyping, DBS should be handled, transported and stored according to the WHO DBS guidelines.

Abstraction and Data Entry

- Data should be abstracted from laboratory submission forms.

Potential biases

- Clinics collecting DBS for EID may tend to be the larger urban or semi-urban sites. Rural sites and smaller sites may not be well represented in the survey.
- Larger, more urban or semi-urban sites that collect DBS for EID may be more likely to make available complex and efficacious PMTCT regimens ; children who themselves received sd-NVP or who were exposed to sd-NVP through their mothers may be under-represented.
- If PMTCT sites contribute most EID specimens, children with "no" or "unknown" ARV exposure may not be well-represented in the survey.

Data Analysis

1. Prevalence of relevant mutations and combinations of mutations, and prevalence of high, intermediate, or low resistance to relevant drug classes and drugs will be calculated for each ARV exposure group, and children < 18 months of age.
2. A separate analysis of prevalence will be performed to evaluate the association the “none” and “unknown” exposure classification with resistance mutations.
3. If the sample size is sufficient, separate analyses will be performed evaluating the association of ARV exposure antepartum, intrapartum and postpartum and the association of various combinations of ARVs on development of resistance mutations.

Potential Public Health Importance of Survey Findings (1)

- Expansion of PMTCT options is likely to lead to changing patterns of DRMs in children infected despite PMTCT exposure
- Identify mutations in HIV-infected infants and children with known and unknown exposure to PMTCT.
- Initial regimens may continue to require modification based on observed DRMs.

Potential Public Health Importance of Survey Findings (2)

- Can potentially identify populations in need of targeted early virologic monitoring (when available) to assess efficacy of first-line regimens

- To assess how time since PMTCT exposure affects observed DRMs (if sample sizes are sufficiently large)

Funding

- Sequencing costs 50-130 USD/test
  - Few labs currently members of the WHO ResNET Lab Network for genotyping DBS; none in Africa
  - CDC Lab charges 50 USD/genotype DBS
  - Monpellier charges 130 USD/genotype DBS
- WHO and PEPFAR will seek funding to support surveys in selected countries.
  - CDC supporting Uganda, PEPFAR COP supporting Malawi
  - WHO supporting Zimbabwe, Swaziland, Cote d'Ivoire, Mozambique, Cambodia ?
- HIVDR-WGs will be encouraged to incorporate the survey into:

  - National Global Fund applications

  - PEPFAR country operation plans

  - Partnerships with academic institutions or NGOs

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